Supplementary material

SM1. GBS Protocol

GBS libraries were prepared and analyzed at the Institute for Genomic Diversity (IGD), according to Elshire et al. (2011), using the enzyme EcoT22I for digestion and creating a library with unique barcodes.

GBS Bioinformatics

The GBS UNEAK analysis pipeline, an extension to the Java program TASSEL (Bradbury et al. 2007), was used to call SNPs from the sequenced GBS library with the following options.

Plugin	Opzione	Valore	Descrizione
UMergeTaxaTagCountPlugin	-m	200000000	Maximum number of tags in the merged TagCount file. (Default: 60,000,000)
UmergeTaxaTagCountPlugin	-c	5	Minimum count required for a tag to be included in the output. (Default: 5)
UmergeTaxaTagCountPlugin	-t		Whether to merge taxa with the same name. "-t n" = do not merge. (Default: merge)
UTagCountToTagPairPlugin	-e	0.03	Error tolerance rate in the network filter. (Default: 0.03)
UMapInfoToHapMapPlugin	-mnMAF	0.05	Minimum minor allele frequency. (Default: 0.05)
UMapInfoToHapMapPlugin	-mxMAF	0.5	Maximum minor allele frequency. (Default: 0.5)
UMapInfoToHapMapPlugin	-mnC	0	Minimum call rate (proportion of taxa covered by at least one tag).
UMapInfoToHapMapPlugin	-mxC	1	Maximum call rate. (Default: 1)
GBSHapMapFiltersPlugin	-mnSCov	0.8	Minimum site coverage (minimum call rate for a SNP to be included). (Default: 0.1)
GBSHapMapFiltersPlugin	-mnTCov	0.1	Minimum *taxon* coverage (minimum SNP call rate for a taxon to be included). (Default: 0.1)
GBSHapMapFiltersPlugin	-mnF		Minimum F value (inbreeding coefficient). Not tested by default.
GBSHapMapFiltersPlugin	-mnMAF	0.01	Minimum minor allele frequency. (Default: 0.0 – no filtering)
GBSHapMapFiltersPlugin	-mxMAF	1	Maximum minor allele frequency. (Default: 1.0 – no filtering)
GBSHapMapFiltersPlugin	-hLD		Specify whether samples should be filtered for high LD. (Default: false)

tbt2vcfPlugin	-mnMAF	0.05	Minimum minor allele frequency. (Default: 0.0)
tbt2vcfPlugin	-mnLCov	0	Minimum locus coverage (proportion of taxa with a genotype). (Default: 0.0)
MergeDuplicateSNP_vcf_Plugin	-ak	3	Maximum number of alleles retained per marker in the population. (Default: 3)

SNP analysis

VCFtools (v0.1.10) (Danecek et al 2011) was used to summarize data, to filter data and to generate input files for PLINK (Purcell et al2007), which were used for MDS (multidimensional scaling). Analyses were visualized using basic plotting functions in R version 2.15.2 (R Development Core Team 2008).

Figure SM2. Factorial Correspondence Analysis (FCA) based on 78 individuals genotyped at 13 STR loci.

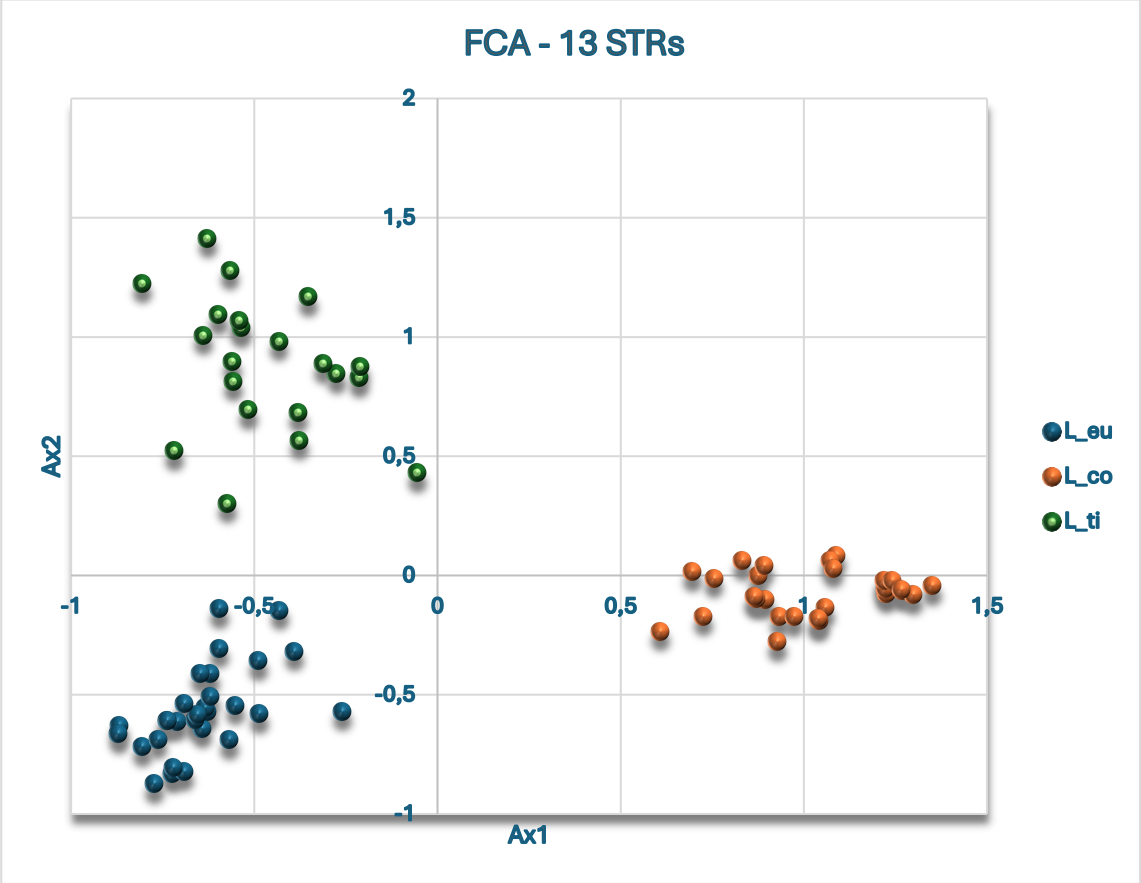


Figure SM3. Identification of the best structure in Adegenet using the function "find cluster" in 78 sample analyzed at 13 STRs and in 71 samples analyzed at 4,472 SNPs. BIC values vs number of clusters **a)** using 13 STRs and **b)** using 4,472 SNPs; **c)** species grouping at K=3 with 13 STRs; **d)** species grouping at K=3 with 4,472 SNPs**; e)** species grouping at K= 2 with 13 STRs: L. *europeus* and L. *timidus* group together; **f)** species grouping at K=2 with 4,472 SNPs: L. *corsicanus* and L. *timidus* group together**.**

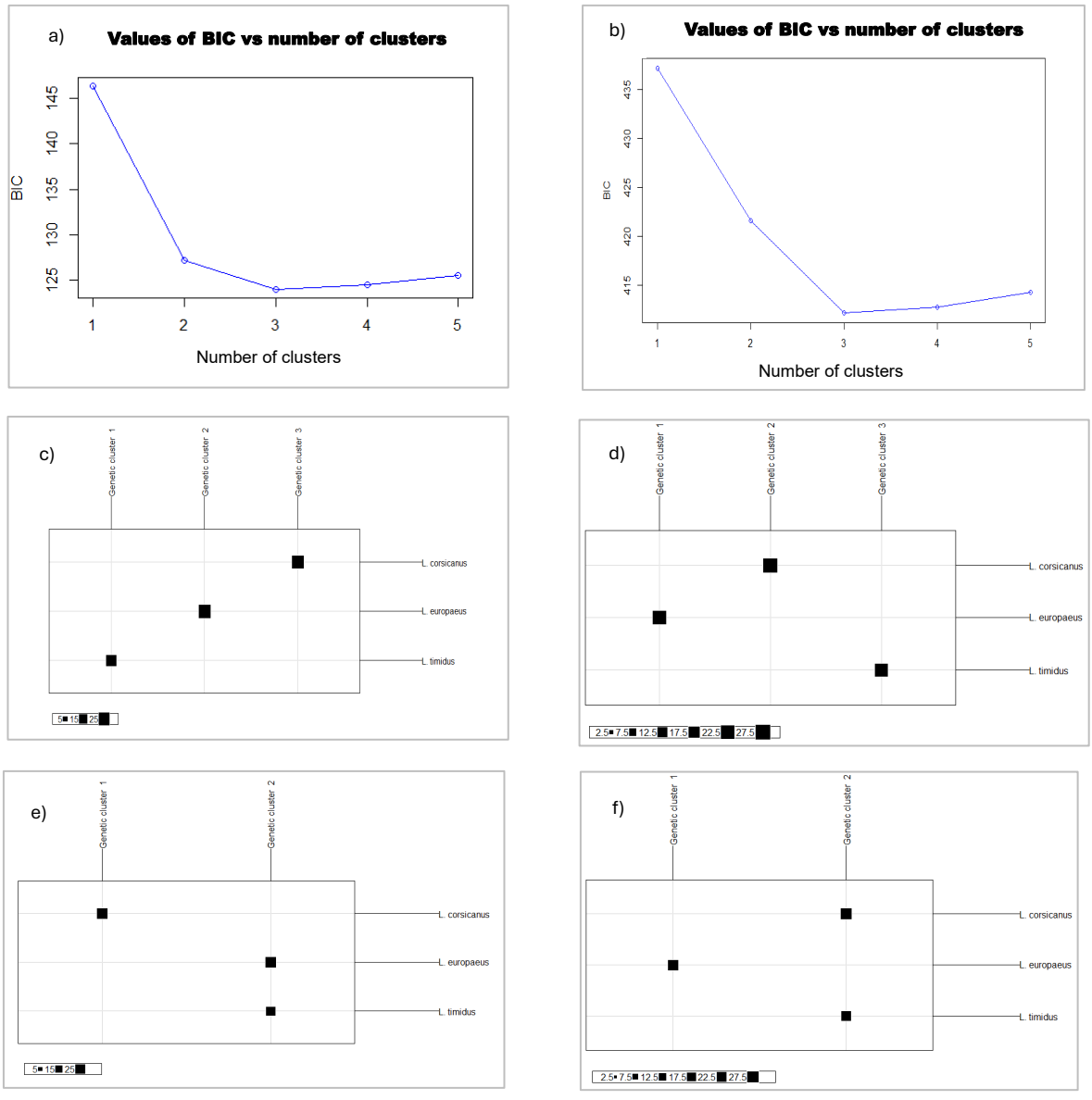
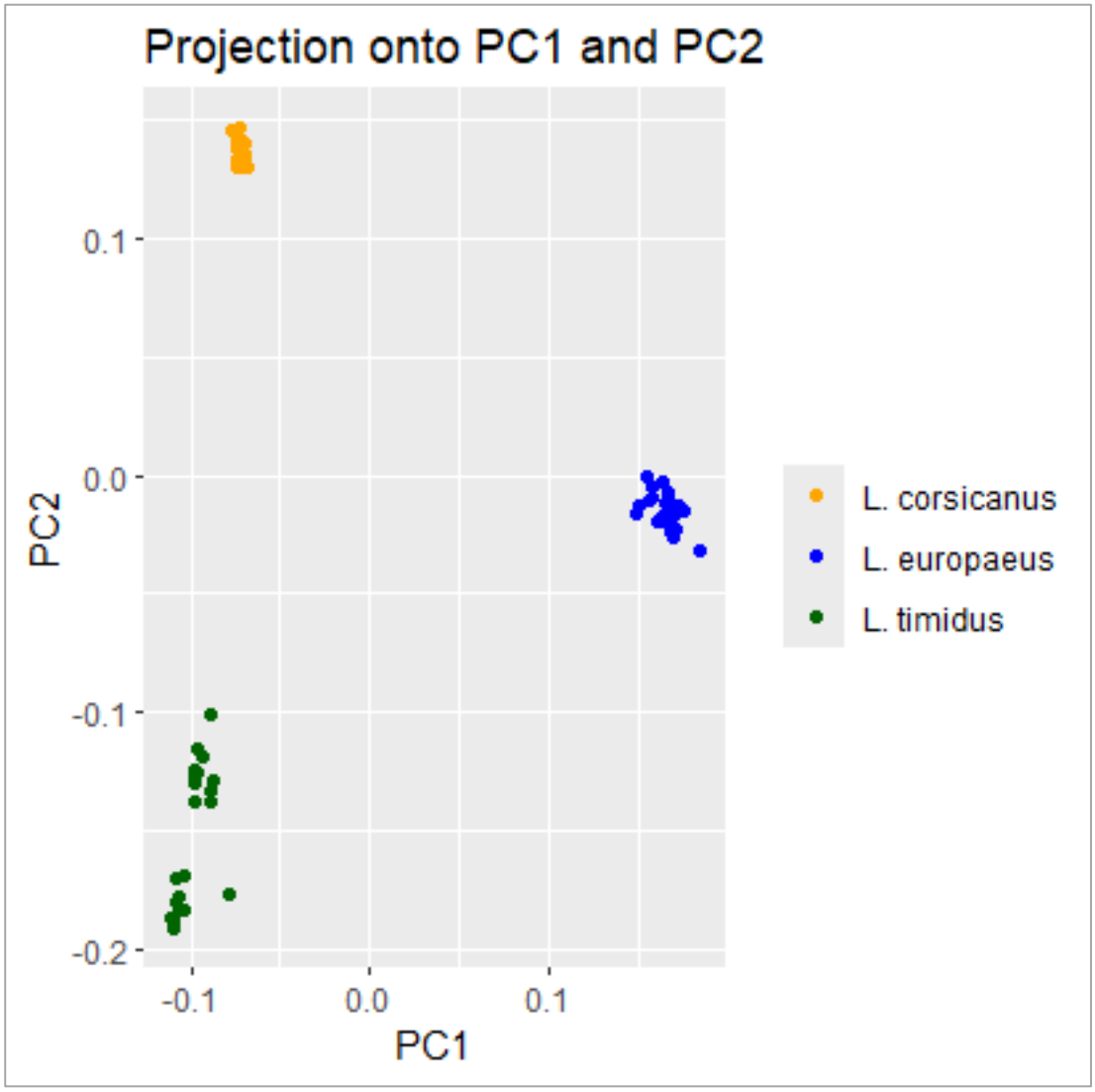


Figure SM4. DAPC based on 71 individuals genotyped at 4,472 SNPs.



References

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., and Mitchell, S.E. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS one **6**(5): e19379.